CLAIMS

1. A method for the detection of glutamate in an experimental sample, comprising the steps of:

a) providing:

i) an experimental sample,

ii) glutamate dehydrogenase,

iii) nicotinamide adenine dinucleotide,

iv) nitro blue tetrazolium chloride, and

v) phenazine methosulfate; and

b) combining said experimental sample, said glutamate dehydrogenase activity, said nicotinamide adenine dinucleotide, said nitro blue tetrazolium chloride, and said phenazine methosulfate under conditions in which said nitro blue tetrazolium chloride is reduced to yield a chromogenic product, and wherein production of said chromogenic product is indicative of glutamate in said experimental sample.

2. The method of Claim 1, further wherein the concentration of glutamate in said experimental sample is quantitated, providing at least two control samples containing known concentrations of glutamate, wherein the quantity of said chromogenic product formed in step b) is proportional to the concentration of glutamate in said samples, and the concentration of glutamate in the experimental sample is determined by constructing a standard curve using said control samples.

3. A method for the detection of ketol-isomerase activity in a sample, comprising the sequential steps of:

a) providing:

i) a sample,

ii) fructose-6-phosphate,

iii) glutamine,

iv) glutamate dehydrogenase,

v) nicotinamide adenine dinucleotide,

vi) nitro blue tetrazolium chloride, and

vii) phenazine methosulfate;

b) combining said sample, said fructose-6-phosphate, and said glutamine under conditions to yield a reaction product comprising glucosamine-6-phosphate and glutamate;

c) inactivating said ketol-isomerase activity; and

d) combining said reaction product with said glutamate dehydrogenase activity, said nicotinamide adenine dinucleotide, said nitro blue tetrazolium chloride, and said phenazine methosulfate under conditions wherein said nitro blue tetrazolium chloride is reduced to yield a chromogenic product, wherein production of said chromogenic product is indicative of ketol-isomerase activity in said sample.

4. The method of Claim 3, wherein said sample comprises a ketol-isomerase selected from the group consisting of fungal ketol-isomerases, bacterial ketol-isomerases, animal ketol-isomerases and plant ketol-isomerases.

5. The method of Claim 3, wherein said sample comprises a crude cell lysate, selected from the group consisting of fungal cell lysates, bacterial cell lysates, animal cell lysates and plant cell lysates.

6. The method of Claim 5, wherein said crude cell lysate is selected from the group consisting of *Aspergillus* cell lysates, *Candida* cell lysates, *Cryptococcus* cell lysates, *Histoplasma* cell lysates, *Pneumocystis* cell lysates, *Rhizopus* cell lysates, *Saccharomyces* cell lysates, *Schizosaccharomyces* cell lysates, *Escherichia* cell lysates, *Staphylococcus* cell lysates and *Pseudomonas* cell lysates.

7. The method of Claim 3, wherein said sample comprises a purified ketol-isomerase.

8. The method of Claim 3, wherein said sample comprises a recombinant ketol-isomerase.

9. The method of Claim 8, wherein said recombinant ketol-isomerase is a recombinant human ketol-isomerase.

10. The method of Claim 3, wherein said inactivating step is selected from the group consisting of boiling and heating to 70°C.

11. The method of Claim 3, further comprising a clarifying step after the inactivating step, wherein said clarifying step is selected from the group consisting of centrifugation, filtration and a combination thereof.

12. A method for the identification of a compound having the ability to inhibit a microbial ketol-isomerase activity, comprising the sequential steps of:

a) providing:

i) microbial ketol-isomerase,

ii) fructose-6-phosphate,

iii) glutamine,

iv) glutamate dehydrogenase,

v) nicotinamide adenine dinucleotide,

vi) nitro blue tetrazolium chloride,

vii) phenazine methosulfate, and

viii) a candidate compound; and

b) preparing a first and a second reaction mixture, wherein said first reaction mixture comprises said microbial ketol-isomerase, said fructose-6-phosphate and said glutamine, and wherein said second reaction mixture comprises said microbial ketol-isomerase, said fructose-6-phosphate, said glutamine and said candidate compound;

c) exposing said first and second reaction mixtures to conditions wherein said microbial ketol-isomerase is capable of producing glucosamine-6-phosphate and glutamate;

d) inactivating said microbial ketol-isomerase;

e) combining said first and second reaction mixtures with said glutamate dehydrogenase, said nicotinamide adenine dinucleotide, said nitro blue tetrazolium chloride, and said phenazine methosulfate under conditions wherein said nitro blue tetrazolium chloride is capable of yielding a chromogenic product, wherein the quantity of chromogenic product produced is proportional to microbial ketol-isomerase activity;

f) comparing ketol-isomerase activities in said first and second reaction mixtures, and

g) identifying a candidate compound having the ability to inhibit said microbial ketol-isomerase activity, wherein the microbial ketol-isomerase activity of said first reaction mixture is greater than the ketol-isomerase activity of said second reaction mixture.

13. The method of Claim 12, wherein said microbial ketol-isomerase comprises a crude microbial cell lysate, selected from the group consisting of fungal cell lysates and bacterial cell lysates.

14. The method of Claim 13, wherein said crude microbial cell lysate is selected from the group consisting of *Aspergillus* cell lysates, *Candida* cell lysates, *Cryptococcus* cell lysates, *Histoplasma* cell lysates, *Pneumocystis* cell lysates, *Rhizopus* cell lysates, *Saccharomyces* cell lysates, *Schizosaccharomyces* cell lysates, *Escherichia* cell lysates, *Staphylococcus* cell lysates and *Pseudomonas* cell lysates.

15. The method of Claim 12, wherein said inactivating step is selected from the group consisting of boiling and heating to 70°C.

16. The method of Claim 12, further comprising a clarifying step after the inactivating step, wherein said clarifying step is selected from the group consisting of centrifugation, filtration and a combination thereof.

17. The method of Claim 12, wherein said compound has the ability to inhibit a microbial ketol-isomerase activity, and further testing said compound for antimicrobial activity using a testing means.

18. The method of ~~Claim~~ 17, wherein said testing means comprises at least one method selected from the group consisting of agar diffusion assays, broth dilution assays, *in vivo* mouse candidosis assays, and *in vivo* mouse aspergillosis assays.

19. The method of Claim 12, wherein said compound preferentially inhibits said microbial ketol-isomerase compared to a second ketol-isomerase, and wherein said method further comprises the steps of:

h) providing a second ketol-isomerase selected from the group consisting of plant ketol-isomerases and animal ketol-isomerases; and

i) preparing a third and a fourth reaction mixture, wherein said third reaction mixture comprises said second ketol-isomerase, said fructose-6-phosphate and said glutamine, and wherein said fourth reaction mixture comprises said second ketol-isomerase, said fructose-6-phosphate, said glutamine and said candidate compound,

j) exposing said third and fourth reaction mixtures to conditions wherein said second ketol-isomerase is capable of producing glucosamine-6-phosphate and glutamate,

k) inactivating said second ketol-isomerase,

l) combining said third and fourth reaction mixtures with said glutamate dehydrogenase activity, nicotinamide adenine dinucleotide, nitro blue tetrazolium chloride, and phenazine methosulfate under conditions wherein said nitro blue tetrazolium chloride is capable of yielding a chromogenic product, wherein production of said chromogenic product is proportional to said second ketol-isomerase activity,

m) comparing said second ketol-isomerase activities in said third and fourth reaction mixtures, and

n) identifying a compound which preferentially inhibits said microbial ketol-isomerase activity compared to said second ketol-isomerase activity.

20. The method of Claim 19, wherein said animal ketol-isomerases are mammalian ketol-isomerases.

21. The method of Claim 20, wherein said mammalian ketol-isomerases are selected from the group consisting of rat ketol-isomerases and human ketol-isomerases.

22. The method of Claim 19, wherein said second ketol-isomerase comprises a cell lysate.

23. The method of Claim 19, wherein said second ketol-isomerase is purified.

24. The method of Claim 19, wherein said second ketol-isomerase is a recombinant ketol-isomerase.

25. The method of Claim 24, wherein said recombinant ketol-isomerase is a recombinant human ketol-isomerase.

26. The method of Claim 19, wherein said inactivating step is selected from the group consisting of boiling and heating to 70°C.

27. The method of Claim 19, further comprising a clarifying step after the inactivating step, wherein said clarifying step is selected from the group consisting of centrifugation, filtration and a combination thereof.

28. The method of Claim 19, wherein said compound preferentially inhibits said microbial ketol-isomerase activity compared to a second ketol-isomerase activity, and further testing said compound for antimicrobial activity using a testing means.

29. The method of Claim 28, wherein said testing means comprises at least one method selected from the group consisting of agar diffusion assays, broth dilution assays, *in vivo* mouse candidosis assays, and *in vivo* mouse aspergillosis assays.

Add a5